

# Experimental evaluation of agonistic behaviour, chemical communication, spacing, and intersexual associations of the Eastern Red-backed Salamander (*Plethodon cinereus*) near its northern range limit

N. Rollinson and D. Hackett

**Abstract:** Territorial behaviour is the use of aggression and advertisement in the defense of a fixed area. In the Eastern Red-backed Salamander (*Plethodon cinereus* (Green, 1818)), territoriality is prevalent in Virginia, USA, near the southern extent of its range, but few studies have examined whether northern populations exhibit territorial behaviour. We tested for some prerequisites of territorial behaviour in *P. cinereus* at the northern extent its range, near North Bay, Ontario, Canada. In laboratory trials, we found that adult male *P. cinereus* exhibited a modest degree of aggression, and intruding salamanders attempted to escape the experimental chamber more often than residents. We also found that *P. cinereus* were able to differentiate between familiar and unfamiliar substrates, suggesting pheromonal communication and a potential for territorial advertisement. However, while male–female pairs were common at six sampling sites during the mating season, adult salamanders consistently exhibited a random spatial distribution during the nonmating season. Our study suggests that local *P. cinereus* exhibit low-level aggression but may not defend fixed areas. One possibility is that *P. cinereus* may generally not benefit from territorial behaviour in cooler climates, as evapotranspiration rates will be lower and foraging activity less restricted compared with more southern climates.

**Key words:** territorial behaviour, territoriality, aggression, nose tapping, Eastern Red-backed Salamander, *Plethodon cinereus*, geographic variation, body size, agonistic encounter, threat displays.

**Résumé :** Le comportement territorial consiste en l'utilisation de l'agressivité et de l'annonce pour défendre une zone fixe. Si, chez la salamandre cendrée (*Plethodon cinereus* (Green, 1818)), la territorialité prévaut en Virginie (É.-U.), près de la limite méridionale de l'aire de répartition, peu d'études ont tenté de déterminer si les populations septentrionales présentent un comportement territorial. Nous avons vérifié la présence de certaines conditions préalables au comportement territorial chez *P. cinereus* à la limite septentrionale de son aire, près de North Bay, Ontario (Canada). Dans des essais en laboratoire, nous avons constaté que les *P. cinereus* adultes mâles présentaient un faible degré d'agressivité, et les salamandres intruses dans l'enceinte expérimentale tentaient de s'en échapper plus souvent que les individus résidents. Nous avons également constaté que les *P. cinereus* étaient capables de distinguer les substrats qui leur étaient familiers d'autres substrats, ce qui pourrait indiquer une communication phéromonale et la possibilité d'annoncer la présence du territoire. Cependant, si les couples mâle–femelle étaient répandus en six sites d'échantillonnage durant la saison des amours, les salamandres adultes présentaient uniformément une répartition spatiale aléatoire en dehors de cette saison. Notre étude donne à penser que, si les *P. cinereus* locaux présentent un faible degré d'agressivité, ils pourraient ne pas défendre de zones fixes. Il est possible que le comportement territorial dans des climats frais ne présente généralement pas d'avantages pour *P. cinereus*, puisque les taux d'évapotranspiration y sont plus faibles et l'activité d'approvisionnement moins restreinte par rapport aux climats plus méridionaux. [Traduit par la Rédaction]

**Mots-clés :** comportement territorial, territorialité, agressivité, tapement du museau, salamandre cendrée, *Plethodon cinereus*, variation géographique, taille du corps, rencontre agonistique, attitudes menaçantes.

## Introduction

Territoriality is the use of overt aggression and advertisement in the defense of a fixed area that becomes exclusive with respect to intruders (Brown and Orians 1970). While territoriality can increase energetic expenditure and mortality due to injury or increased exposure to predators, it affords individuals the benefit of priority or exclusive access to shelter, food items, mates, and other resources. It is therefore expected to evolve where these benefits outweigh the costs of maintaining a dominant status

(Brown 1964). For example, when resources are limited and occur in defensible patches, superior competitors stand to benefit by monopolizing these areas (Mathis 1990), thereby leading to the evolution of territorial behaviour.

One interpretation of territoriality is that it represents an extreme or endpoint along a continuum of social interactions and spacing systems (Maher and Lott 1995). In this paradigm, a species or population might exhibit many features of territorial behaviour, such as dominance behaviours and nonoverlapping home ranges, while nevertheless falling short of true territoriality (Brown

Received 2 March 2015. Accepted 29 June 2015.

N. Rollinson\* and D. Hackett. Department of Biology, Nipissing University, 100 College Drive, North Bay, ON P1B 8L7, Canada.

**Corresponding author:** N. Rollinson (e-mail: [njall.rollinson@gmail.com](mailto:njal.rollinson@gmail.com)).

\*Present address: Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, ON M5S 3B2, Canada.

and Orians 1970). Therefore, if the goal is to better understand spacing systems and their underlying ecological and social causes, then it is important to adopt criteria that delineate true territorial behaviour: animals that engage in territoriality should exhibit site fidelity, an ability to defend these sites, a high probability of expelling intruders from their territories, and a mechanism of territorial advertisement (Gergits 1982; Mathis et al. 1995). As a result of these behaviours, territorial animals should also exhibit a uniform spatial distribution in the field, with respect to the distribution of resources, at some point during the growing and (or) reproductive season (Brown and Orians 1970). Having adopted this definition, we can ask how populations and species differ in their extent of territorial behaviour, such that we are better positioned to understand what ecological or social factors might drive the evolution of territoriality and spacing systems more generally.

The Eastern Red-backed Salamander (*Plethodon cinereus* (Green, 1818)) is a model organism for the study of territoriality and social systems. Populations of this species at Mountain Lake Biological Station (MLBS), Virginia, USA, have been the subject of intense behavioural research for decades (Jaeger 1980; Prosen et al. 2004; Liebgold 2014). At MLBS, *P. cinereus* use complex chemical communication systems (Jaeger 1986; Mathis and Simons 1994; Kohn et al. 2013), exhibit sophisticated combat tactics in agonistic encounters (Jaeger 1981, 1984; Jaeger and Schwarz 1991), and form intersexual associations and co-defend territories with a member of the opposite sex (Gillette et al. 2000). While the majority of research on the territorial behaviour of *P. cinereus* has been conducted at one location, MLBS, geographic variation in the territorial and social behaviour of plethodontid salamanders has been documented (Nishikawa 1985; Wiltenmuth and Nishikawa 1998; Quinn and Graves 1999; Maerz and Madison 2000). Jaeger and Forester (1993) emphasized that studies on *P. cinereus* from an array of geographic locations would be extremely valuable in further understanding territoriality and the mechanisms driving the evolution of such complex behaviours and social systems in this and other species.

Territoriality in *P. cinereus* seems to be favoured at MLBS and in some other areas (e.g., Maerz and Madison 2000; Liebgold 2014), as population densities can be very high (Mathis 1991) and because the resources on which *P. cinereus* depend become periodically limited throughout the growing season (Jaeger 1980). An abundance of suitable prey, such as small, soft-bodied insects, can normally be found in the leaf litter on the forest floor where these creatures live. As the leaf litter dries between rainy periods, *P. cinereus* are forced to take refuge under moist cover objects, such as rocks and logs, where they avoid desiccation and where insect prey can still be found in relative abundance (Jaeger 1980). In territorial populations, *P. cinereus* aggressively defend these cover objects against intruding salamanders (Jaeger 1981, 1984; Jaeger et al. 1982; Mathis 1991). The defense of territories by *P. cinereus* should ensure that they retain an environmentally stable area that is exclusive of other salamanders (except potential mates and juveniles) and that contains an abundance of suitable prey.

It is not known whether territoriality is prevalent throughout the range of this species. For example, Quinn and Graves (1999) show that *P. cinereus* in a Michigan population may not be territorial. Instead, individuals from northern Michigan often aggregate in the field and under laboratory conditions. The observed differences in behaviour between MLBS and Michigan could be due to a greater availability of high-quality habitat and perhaps a lower population density in Michigan, which would presumably reduce the benefit of monopolizing resources in a single area. Furthermore, Maerz and Madison (2000) report differences in territorial behaviour between populations of *P. cinereus* at sites no more than 30 km apart near the New York and Pennsylvania border. They attributed this phenomenon to predictable differences in foraging success based on food availability. At a site with low-quality

food (i.e., chitinous insects), *P. cinereus* appeared to defend fixed food-based territories. However, at a site with high-quality food (i.e., soft-bodied invertebrates), *P. cinereus* did not appear to defend territories. These two studies suggest that geographic variation in the territorial behaviour of *P. cinereus* might exist over relatively short distances, such that it would be valuable to assess the extent of territoriality in other populations. Given that a lack of territoriality has been documented in Michigan, near the northern extent of this species' range (Quinn and Graves 1999), it might be especially valuable to investigate further the extent of territoriality in very northern populations.

Quantifying territoriality can be difficult for small, cryptic animals; the most practical and productive approach for salamanders has been to test predictions of territoriality in specific contexts (Mathis et al. 1995, 1998). In the present study, we use four different experiments to examine specific aspects of the spacing system in a population of *P. cinereus* near the northernmost extent of this species' range, near North Bay, Ontario, Canada (46°19'00"N, 79°27'58"W). First, we investigate whether salamanders react aggressively towards individuals of the same sex and whether residents have a high probability of expelling intruders from their "territories". Next, we investigated whether individuals in local populations are able to detect novel chemical stimuli (e.g., pheromones of conspecifics), a necessary prerequisite in populations that use territorial advertisement. Third, we investigated the spatial distribution of *P. cinereus* in the field, thereby determining whether populations tend towards a uniform distribution as at MLBS (e.g., Mathis 1991), a clumped distribution as in Michigan (Quinn and Graves 1999), or a random distribution. Finally, we examine pairing patterns in the field to determine whether intersexual pairs occur more often than intrasexual pairs. Although site fidelity is an important aspect of territoriality, testing this was beyond the scope of our study.

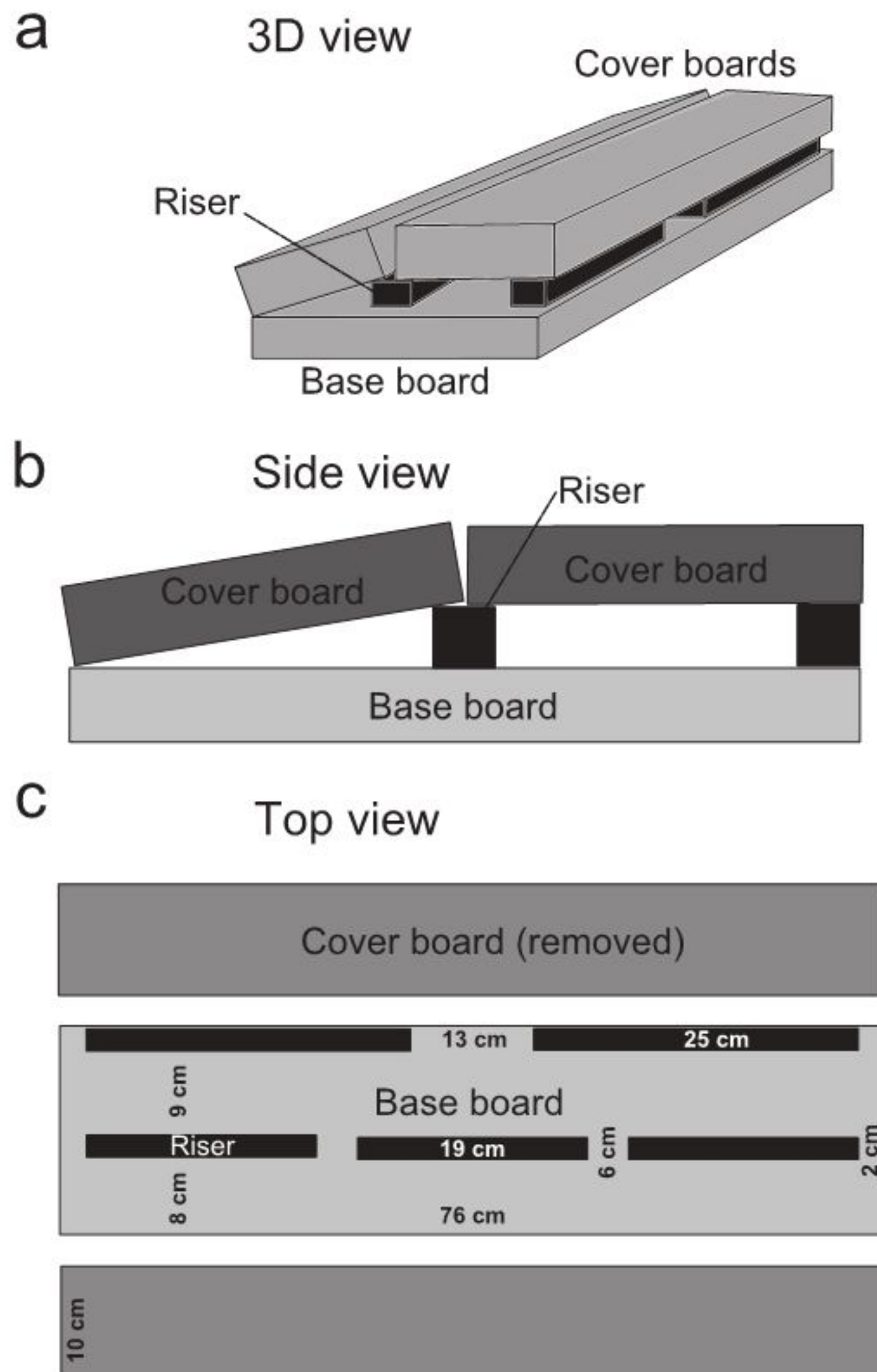
## Materials and methods

The Ontario Ministry of Natural Resources and Forestry (MNRF) Terrestrial Wildlife Assessment Program established permanent sampling plots in the North Bay region to monitor *P. cinereus*. Each plot is located in a specific forest type and consists of a 500 m transect with a buffer zone of at least 100 m from any different forest types and major roads. Each plot contains 70 modified Davis boards, which are artificial cover objects used to monitor *P. cinereus* (Figs. 1a–1c), interspersed at approximately 10 m intervals (Sugar et al. 2000). Davis boards occupy an area of 0.152 m<sup>2</sup> (Fig. 1c), which is similar to the mean size of areas occupied by adult *P. cinereus* at MLBS, which is approximately 0.163 m<sup>2</sup> (Mathis 1991). We used six of these long-term research plots in the present study. All plots were in old deciduous forest stands, predominantly stands of sugar maple (*Acer saccharum* Marsh.) mixed with softwood species (e.g., pine (genus *Pinus* L.), spruce (genus *Picea* A. Dietr.), fir (genus *Abies* Mill.)), aspen (genus *Populus* L.), and birch (genus *Betula* L.). The minimum and maximum distances between two plots was 5 and 47 km, respectively (Table 1).

## Aggression and the expulsion of intruders

Fifty adult male *P. cinereus* (snout-to-vent length (SVL) range: 33–47 mm) were collected between 15 July and 13 October 2002. The salamanders were collected from various MNRF sampling plots in the North Bay area (Table 1). They were housed in plastic containers that were approximately 23 cm × 12 cm × 4 cm. The containers were lined with moist filter paper and all individuals were kept in perpetual darkness at 18–23 °C to mimic the lack of light under natural cover objects. All salamanders were kept for approximately 45 days prior to testing and were maintained on *D. melanogaster*. Agonistic trials were conducted on 18 August 2002 (*n* = 6 trials), 19 August 2002 (*n* = 6 trials), 18 September 2002 (*n* = 5 trials), 19 September 2002 (*n* = 3 trials), and 9 December 2002 (*n* = 5 trials). Each salamander was tested only once, either as a resident

**Fig. 1.** A Davis board. (a) A three-dimensional view. (b) A lateral view from the ground level, with the middle compartment visible. (c) A view from above, with cover boards removed and placed on either side of the base board so that the entire middle compartment is visible.



or as an intruder. Given that salamanders were collected and tested over a range of dates, we caution of our implicit assumption that collection and test date had little or no effect on the behaviours expressed and on the outcome of trials (for evidence against this assumption see Jaeger 1979; Wise 1995).

Five days before testing, future residents were placed into larger 33 cm × 20 cm × 14 cm plastic enclosures, but they were otherwise maintained under the same conditions as before. The encounters consisted of removing the future intruder, which was matched to the resident for SVL ( $\pm 3$  mm), from its original enclosure, quickly placing it into the resident's enclosure, and covering it with an opaque habituation lid. The resident was similarly removed, handled, and replaced into its enclosure and covered with an opaque habituation lid. After 5 min, the habituation lids were removed and the ensuing interaction was observed for 25 min. The amount of time each salamander spent in "all-trunk-raised" (ATR) and "edge" behaviours was recorded and the number of times each salamander delivered a bite was noted. ATR is a threat posture (Jaeger 1984; Jaeger and Schwarz 1991); biting is an aggressive act and occurs when one salamander bites the other salamander (Jaeger 1984). "Edge" behaviour occurs when a salamander presses its nose against the side of the container and (or) climbs the wall of the combat container in an attempt to escape; "edge" is considered to be an escape behaviour (Wise 1995; Jaeger et al.

1995). An increasing time spent in ATR postures and an increasing number of bites corresponds with an increasing level of aggression towards a conspecific, whereas an increasing "edge" behaviour corresponds with a greater propensity to escape the area (Jaeger et al. 1995). All tests were conducted at room temperature (18–20 °C) under a light intensity of less than 10 foot candles (1 foot candle = 10.76391 lx). We tested whether residents spent more time in ATR, delivered more bites, and spent less time in "edge" behaviour using a Wilcoxon sign-rank test.

#### Capacity for territorial advertisement

We tested whether *P. cinereus* from northern Ontario can differentiate between self-marked substrates and those marked by conspecifics (Mathis et al. 1998). Size at maturity is not known for these populations of salamanders, although data from Virginia (Gillette et al. 2000) and Baltimore, USA (Sayler 1966), suggest that individuals are sexually mature when they reach 32 mm SVL. Thus, 12 adult male salamanders with SVL  $\geq 32$  mm were collected from MNR sampling plots from 10 to 13 October 2002; the salamanders were sexed using the candling method described by Gillette and Peterson (2001). The salamanders were housed in a laboratory at Nipissing University (North Bay, Ontario, Canada) in 23 cm × 12 cm × 4 cm containers lined with moist filter paper at 17–24 °C and kept in perpetual darkness for 90 days prior to testing to mimic life underneath cover objects and in burrows. They were fed 10–20 fruit flies (*Drosophila melanogaster* Meigen, 1830) every 2–3 days and their filter paper was changed every 10 days. Four days prior to the beginning of the trials, we changed each individual's filter paper and each individual was fed 20 *D. melanogaster*. Previous studies have shown that territorial individuals are able to establish territories in 4 days (Jaeger 1981).

Trials consisted of removing the test salamander from its chamber and replacing it either into its own chamber or into the empty container of a conspecific. The test salamander was then placed under an opaque habituation lid for 3 min; the lid was then removed and the salamander was allowed to explore the test chamber for 15 min. Each salamander was tested twice: once in its own container and once in that of a conspecific. Thus, 12 salamanders were tested on day 4; the same 12 were tested again on day 8, but under the opposite test condition, with test condition for a given salamander selected haphazardly with respect to day. The number of times the salamander pressed its nasolabial cirri to the substrate (Jaeger 1981; "nose-tapping") was recorded in each trial. Nose-tapping is used by *P. cinereus* to convey chemical information from the substrate to the vomeronasal organ (Dawley and Bass 1989); the frequency of nose-tapping to a given substrate should reflect a salamander's level of interest in the odors and (or) pheromones on that particular substrate (Jaeger 1986; Mathis and Simons 1994; Simons et al. 1994; Mathis et al. 1998). Nose-tap data were analyzed using a Wilcoxon sign-rank test. All tests were conducted at room temperature (18–20 °C) under a light intensity of less than 10 foot candles.

#### Spatial distribution in the field

Observational data from MNR sampling plots were used to examine the spatial distribution of individuals in the field. As part of standard MNR protocol, each plot was visited four times (at 2-week intervals) between 17 May and 14 July 2002; this sampling scheme avoided the breeding season, which occurs in the fall. Plot visits consisted of searching the middle compartment of each Davis board (Figs. 1a–1c), as well as under each board, and noting the body size of salamanders (small: <25 mm body length; medium-sized: 25–65 mm body length; large: >65 mm body length; Sugar et al. 2000). Size at maturity is not known for these populations of salamanders, although data from Virginia (Gillette et al. 2000) and Baltimore (Sayler 1966) suggest that individuals are sexually mature when they reach 32 mm SVL. In the present study, all "large" salamanders observed during plot visits were assumed to be

**Table 1.** The number of adult Eastern Red-backed Salamanders (*Plethodon cinereus*) collected for agonistic trials and surveyed for intersexual associations at each study plot.

Plot identification	Predominant tree species	No. collected for agonistic trials	No. surveyed for intersexual associations
A	Mixedwood*	4	56
B	Mixedwood*	9	41
C	Tolerant hardwood†	9	37
D	Tolerant hardwood†	26	42
E	Tolerant hardwood†	2	39
F	Intolerant hardwood‡	0	29

\*Sugar maple (*Acer saccharum*); species of pine (*Pinus* sp.) and spruce (*Picea* sp.); balsam fir (*Abies balsamea* (L.) Mill.).

†*Acer saccharum*.

‡Aspen (*Populus* sp.); paper birch (*Betula papyrifera* Marshall).

adults capable of potentially holding territories. “Small” and “medium-sized” salamanders were omitted from the spatial analysis, as territorial adults at MLBS tolerate juvenile intrusion into their territories (Jaeger et al. 1995) and because juveniles do not appear to establish territories (Mathis 1991).

Salamanders can be found both under and within Davis boards (Figs. 1a–1c); however, individuals can be easily overlooked when they are situated in the leaf litter on which the boards are placed. For this reason, we considered salamander presence–absence data from between the boards, but we omitted presence–absence data collected from under the boards. We could therefore assume that each sampling visit at each plot represented an equal sampling effort where a systematic search of 70 cover objects of similar quality was performed. To estimate the spatial distribution of *P. cinereus* in the field, we calculated Morisita’s index of dispersion (Morisita 1962) for each plot of 70 Davis boards for each of the four sampling visits. We standardised the index following Smith-Gill (1975), such that the index varied between –1.0 and 1.0, with negative values suggesting a uniform distribution and positive values suggesting an aggregated distribution. Ninety-five percent confidence intervals around zero are between 0.5 and –0.5, such that index values falling within this range suggest a random spatial distribution.

### Intersexual associations

Six MNRF salamander sampling plots were surveyed between 10 and 13 October 2002 (mating season) (Table 1). Salamanders were observed by searching Davis boards that had previously been placed at the plots (Figs. 1a–1c). All salamanders having a SVL  $\geq 32$  mm were considered to be sexually mature (Sayler 1966; Gillette et al. 2000) and were sexed using the candling method described by Gillette and Peterson (2001). A “pair” was defined as two mature salamanders found within 30 cm of each other on the horizontal plane, which is standard procedure for examining paired salamanders (Gillette et al. 2000; Peterson 2000; Jaeger et al. 2002). A  $\chi^2$  goodness-of-fit test on observed and expected frequencies of inter- and intra-sexual pairs was performed to determine whether or not there were significantly more male–female pairs than expected by chance in the mating season.

## Results

### Aggression and the expulsion of intruders

There were no significant differences between residents and intruders in the number of bites delivered ( $Z = -0.480$ ,  $n = 25$  pairs,  $P = 0.631$ ; Fig. 2a) or in the amount of time either party spent in ATR threat postures ( $Z = -1.42$ ,  $n = 25$  pairs,  $P = 0.156$ ; Fig. 2b). However, intruders spent significantly more time in “edge” behaviour than residents ( $Z = -2.06$ ,  $n = 25$  pairs,  $P = 0.040$ ; Fig. 2c).

### Capacity for territorial advertisement

*Plethodon cinereus* delivered significantly more nose taps to substrates marked by conspecifics ( $6.08 \pm 1.23$  nose taps, mean  $\pm$  SE)

than to self-marked substrates ( $3.08 \pm 0.633$  nose taps, mean  $\pm$  SE) in experimental trials ( $Z = -2.50$ ,  $n = 12$ ,  $P = 0.012$ ; Fig. 2d). Individuals were also observed tapping fecal pellets during these trials and during agonistic trials.

### Spatial distribution in the field

In a given visit to a sampling plot, the total number of large salamanders observed across all Davis boards ranged from 2 to 76 individuals. With 70 cover objects, the minimum sample size of salamanders needed to observe a uniform distribution is 23 individuals and all individuals would need to be found under different cover objects to infer a uniform distribution. This minimum sample size was achieved in 17 of all 24 plot visits. *Plethodon cinereus* exhibited a random spatial distribution in 16 of these 17 visits and a uniform distribution in only 1 of these visits (Figs. 3a, 3b). To detect an aggregated distribution, only two salamanders would have to be observed, both together. An aggregated spatial distribution was never observed (Figs. 3a, 3b).

### Intersexual associations

Of the 244 adult salamanders examined in our survey during the mating season of 2002, 9 adults were involved in a mixed-sex trio of individuals (3.7% of surveyed adults). These mixed-group individuals were excluded from further analysis. We found 58 salamanders in intersexual pairs (female–male pairs) that were exclusive of other adults (23.8% of surveyed adults), but only 10 salamanders were paired intrasexually (4.1% of surveyed adults), with three female–female pairs and with two male–male pairs. Significantly more intersexual adult pairs than intrasexual adult pairs were therefore observed in the field ( $\chi^2_{[2]} = 14.98$ ,  $\alpha = 0.05$ ,  $P < 0.001$ ; Table 2).

## Discussion

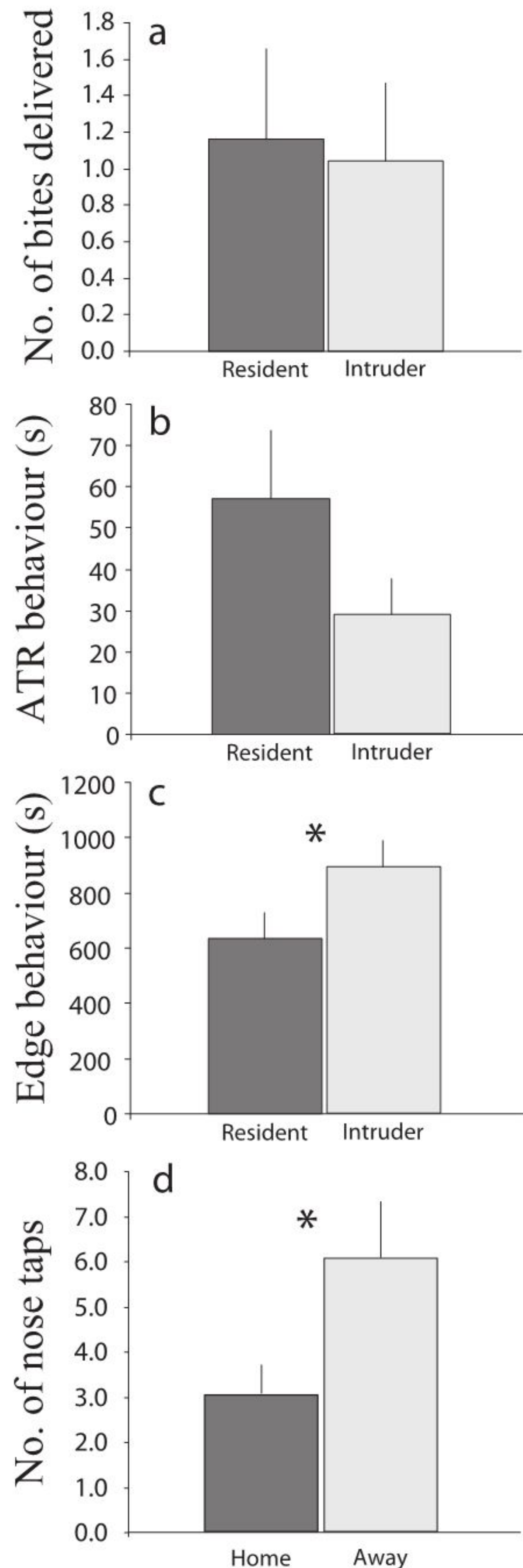
The present study communicates four major findings. First, male *P. cinereus* may act aggressively towards unfamiliar individuals, engaging threat posturing and even biting conspecifics. Relatedly, when placed in the “territory” of another individual, the intruding salamander engaged in escape behaviour for significantly longer than the resident. Second, *P. cinereus* were able to differentiate between familiar and unfamiliar substrates, suggesting that chemical communication among conspecifics is possible in this population. Third, the predominant spatial distribution of adult *P. cinereus* in the field was random, even when the number of observed salamanders exceeded the number of potential cover objects. Finally, we found that intersexual associations in the field were common during the mating season, whereas intrasexual associations were rare. Below we discuss the extent to which these behaviours are consistent with those observed in other populations and we explore the extent to which our observations are consistent with territorial behaviour.

**Fig. 2.** Behaviours exhibited by Eastern Red-backed Salamanders (*Plethodon cinereus*) during laboratory trials. Values in each panel are means  $\pm$  SE and the asterisk (\*) indicates a statistically significant difference. (a) Number of bites delivered in agonistic trials. (b) Number of seconds spent in the aggressive “all-trunk-raised” posture in agonistic trials. (c) Number of seconds spent in “edge” behaviour in agonistic trials. (d) Number of nose taps performed while exploring substrates marked by conspecifics (“away”) versus self-marked substrates (“home”) in pheromone trials.

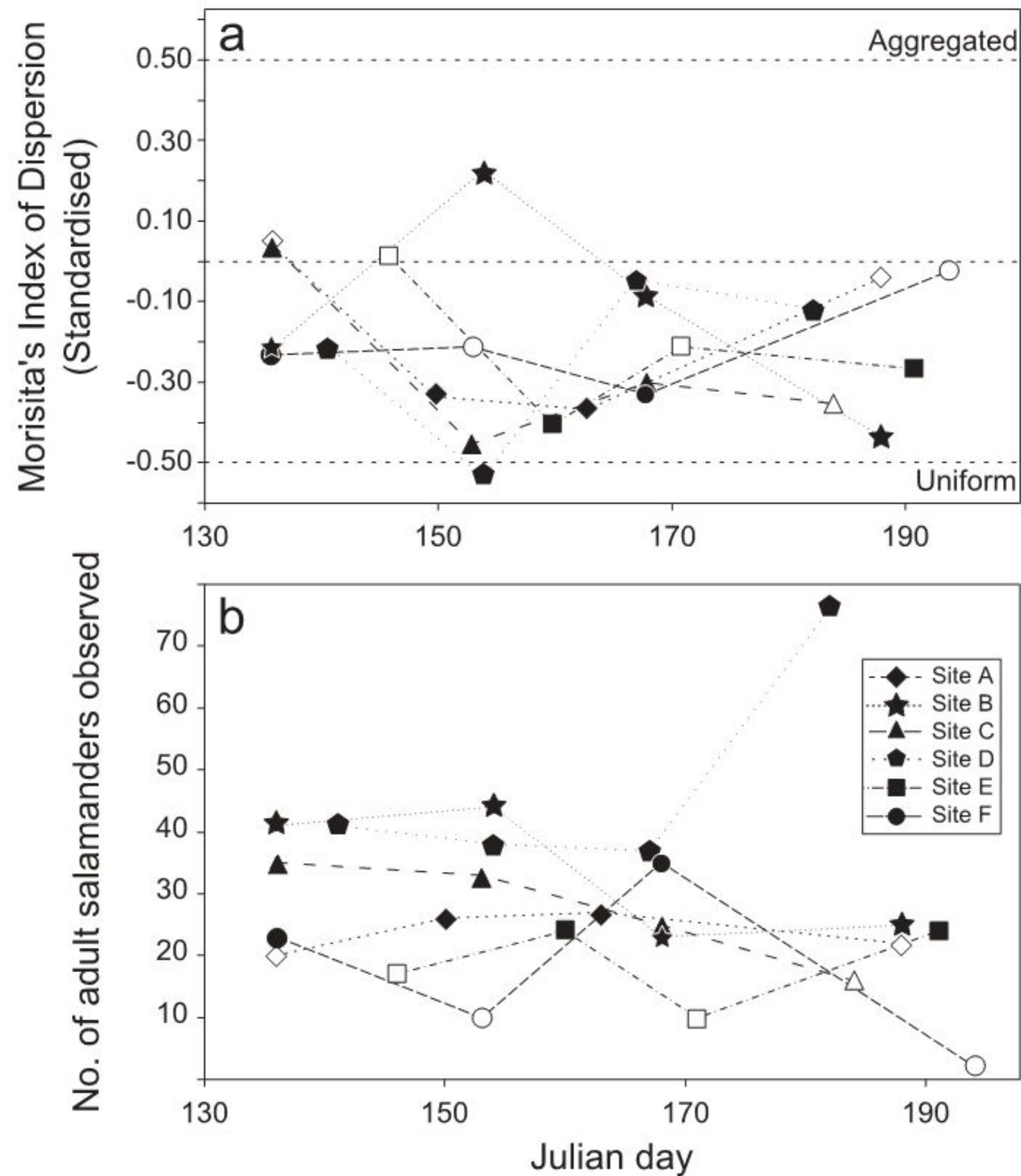
#### Aggression and the expulsion of intruders

We observed that *P. cinereus* from Ontario engaged in aggressive behaviour (ATR and biting), but previous occupancy of an area did not correspond with significantly more aggressive behaviour. This is in contrast to the findings of several studies performed on individuals from territorial populations, where ATR behaviour is typically observed more frequently in territorial residents (e.g., Jaeger 1984; Jaeger and Schwarz 1991). Interestingly, while there was a trend in the present study for resident salamanders to exhibit more ATR behaviour than intruding salamanders (Fig. 2b), resident salamanders spent only 3.8% of total trial time in ATR displays. In many other populations, salamanders subjected to similar agonistic trials appeared to spend much more time in ATR. For example, Wise (1995) performed agonistic trials on two different Virginian populations at five different times of the year, and even during the time of year where salamanders were least aggressive, she found that ATR comprised between 42% and 46% of trial time (Fig. 5.2 in Wise 1995). Jaeger (1984) also found that resident *P. cinereus* from MLBS spent about 38.0% of trial time in ATR (Table 4 in Jaeger 1984); similar proportions have been found for *P. cinereus* in Ohio (Gall et al. 2003; Hickerson et al. 2004). Even for the Southern Red-backed Salamander (*Plethodon serratus* Grobman, 1944), a species for which territoriality is generally suspected (Mathis et al. 1995, 1998), resident *P. cinereus* spent 16.2% of trial time in ATR (Table 1 in Mathis et al. 1998). More broadly, aggressive behaviour is indeed present in Ontario *P. cinereus*, but limited comparison suggests that individuals from local populations do not spend as much time in threat displays as other populations, particularly those that are known to be territorial. Further study is warranted to confirm this suggestion, however, as observer bias or differences in sampling design might also have contributed to this qualitative difference. Finally, we note that biting has been observed in a wide variety of salamanders from the genus *Plethodon* (Mathis et al. 1995) and it is therefore not unexpected to observe this behaviour in Ontario (Fig. 2a). Furthermore, while resident salamanders did not deliver more bites in the present study, biting rarely differs between residents and intruders in agonistic trials (e.g., Jaeger 1984; Mathis et al. 1998), presumably because this behaviour occurs infrequently.

In territorial populations, territorial residents have an inherent “resident advantage” in symmetric contests (Krebs 1982). Residents are more likely to win agonistic encounters in their own territories, which means that intruders are more likely to be expelled from the resident’s territory (Davies 1978; Krebs 1982). In the present study, we observed that intruders engaged in more escape behaviour than residents. If we adopt the assumption that escape behaviour is both submissive and predictive of expulsion in the wild, then our data are consistent with the “resident advantage” hypothesis, which is characteristic of territorial populations (Davies 1978; Barnard and Brown 1982; Krebs 1982; Waage 1988). However, as illustrated above, territorial residents did not engage in more aggressive behaviour than intruders and little overall trial time was spent in threat displays. In the absence of a complementary finding that territorial residents behaved in a threatening manner, the finding that intruders engaged in more escape behaviour can be attributed to a desire by intruding salamanders to escape a novel environment. In this interpretation, the escape



**Fig. 3.** Sample size and spatial distribution of Eastern Red-backed Salamanders (*Plethodon cinereus*) at six sampling plots as a function of time. (a) Morisita's index was standardised following Smith-Gill (1975): 95% confidence intervals (CI) for a random distribution span -0.5 to 0.5, such that any index value below -0.5 or above 0.5 would allow statistically robust inference of a uniform or aggregated spatial distribution, respectively. Open symbols indicate sampling events where less than 23 adults were observed, such that sample size was not large enough to detect a uniform spatial distribution. (b) Number of adult salamanders encountered in each plot visit; 70 Davis boards were sampled each visit.



**Table 2.** Chi-square goodness-of-fit test on observed and expected frequencies of inter- and intra-sexual pairs of Eastern Red-backed Salamanders (*Plethodon cinereus*) found in the 2002 mating season in northern Ontario.

	Frequency of paired individuals	Proportion of individuals in pairs	Expected			Observed frequencies of pairs
			Proportions of paired individuals	Frequencies of individuals	Frequencies of pairs	
♂	33	0.486	0.236	16.0	8.00	2 (♂♂)
♀	35	0.514	0.264	18.0	9.00	3 (♀♀)
♀+♂			0.500	34.0	17.0	29 (♀♂)
Sum	68	1.00	1.00	68.0	34	34

Note:  $\chi^2_{(2)} = 14.98, P < 0.001$ .

behaviour that we observed is not submissive, but is rather brought on by unfamiliarity with a new environment. In sum, our agonistic trials do not provide strong evidence of “resident advantage”.

**Capacity for territorial advertisement**

We observed that salamanders nose-tapped more often to substrates that were previously occupied by a conspecific compared with their own substrate. We therefore infer that *P. cinereus* in

northern Ontario are able to discriminate between familiar and novel substrates. While our findings do not necessarily imply that individuals were responding to pheromones left on substrates by conspecifics, this interpretation would be consistent with the nature of the chemical communication system documented in other populations. Previous studies with *P. cinereus* show that nasolabial grooves convey chemicals to the vomeronasal organ (Dawley and Bass 1989), and this ultimately allows an individual to assess the status of conspecifics, such as sex and reproductive status (Dantzer

and Jaeger 2007). Nevertheless, even if *P. cinereus* in northern Ontario do have a potential mechanism of territorial advertisement, our observations do not necessarily imply territoriality in local *P. cinereus*. For example, there is little clear evidence of territorial behaviour in Mole Salamanders (genus *Ambystoma* Tschudi, 1838), but members of this group are also able to detect conspecifics using nose-tapping (Mathis et al. 1995).

### Spatial distribution in the field

A uniform spatial distribution in the field is an emergent property of territoriality, resulting from site fidelity and the defense of a fixed space that is exclusive to intruders (Brown and Orians 1970). In the present study, the predominant spacing pattern was random (Figs. 3a, 3b). This pattern differs from spatial distributions observed in Michigan, where adult *P. cinereus* are often aggregated (Quinn and Graves 1999), and from MLBS, where individuals exhibit uniform spatial distributions (Jaeger 1979; Mathis 1991; Jaeger et al. 1995). In fact, Jaeger et al. (1995) also used Morisita's standardised index of dispersion at a site at MLBS and found very strong evidence of a uniform spatial distribution for adults, where index values approached the minimum value of  $-1.0$  (cf. Fig. 3a).

The extent of territorial behaviour may change across the growing season and spatial distributions might therefore also be expected to experience a temporal dynamic. For instance, at MLBS, groups of several adult *P. cinereus* may be common in the spring, but in the summer, cover objects generally harbor only one individual (Jaeger 1979), which presumably reflects increased territorial behaviour as the forest floor dries into the summer. In the present study, a random spatial distribution was temporally consistent and there was no qualitative trend for spatial distributions to change predictably from spring to early summer (Fig. 3a). It is worth noting, however, that Morisita's standardised index of dispersion was usually less than zero in the present study, indicating that all plots tended more towards a uniform spatial distribution than towards a clumped distribution. Given that distributions arise from a variety of coincident and complimentary behaviours, it is difficult at the present time to speculate on the proximate reason why spatial distributions of *P. cinereus* in Ontario differ from both Michigan and MLBS.

### Intersexual associations

As the summer progressed into the fall breeding season, we found that adult salamanders were likely to associate with members of the opposite sex in the field. Interestingly, only 3 of the 29 intersexual pairs of adult salamanders were found in the presence of another adult salamander (i.e., 3 mixed-sex trios were observed compared with 29 male–female pairs), such that these pairs were essentially exclusive of other adults. In territorial populations at MLBS, intersexual pairs occupying the same territory preferentially associate with their partner over a stranger of the opposite sex and intersexual pairs engage in sexual coercion and even appear to co-defend territories against intruders (Gillette et al. 2000; Jaeger et al. 2002; Lang and Jaeger 2000; Prosen et al. 2004). However, intersexual associations during the breeding season are not necessarily associated with territoriality (Brown and Orians 1970), especially in the absence of evidence that a uniform spatial distribution prevails during the nonbreeding season. For instance, Ovaska (1988) found that Western Red-backed Salamanders (*Plethodon vehiculum* (Cooper, 1860)) exhibited random spatial distributions under laboratory conditions and that males were aggressive only during the height of the breeding season, but not at other times of year (but see Wise 1995). The suggestion is that aggression in the absence of territoriality may result in exclusive pairing during the mating season, such that the presence of intersexual pairs in the present study does not necessarily suggest that social interactions between mating pairs are similar to those observed at MLBS.

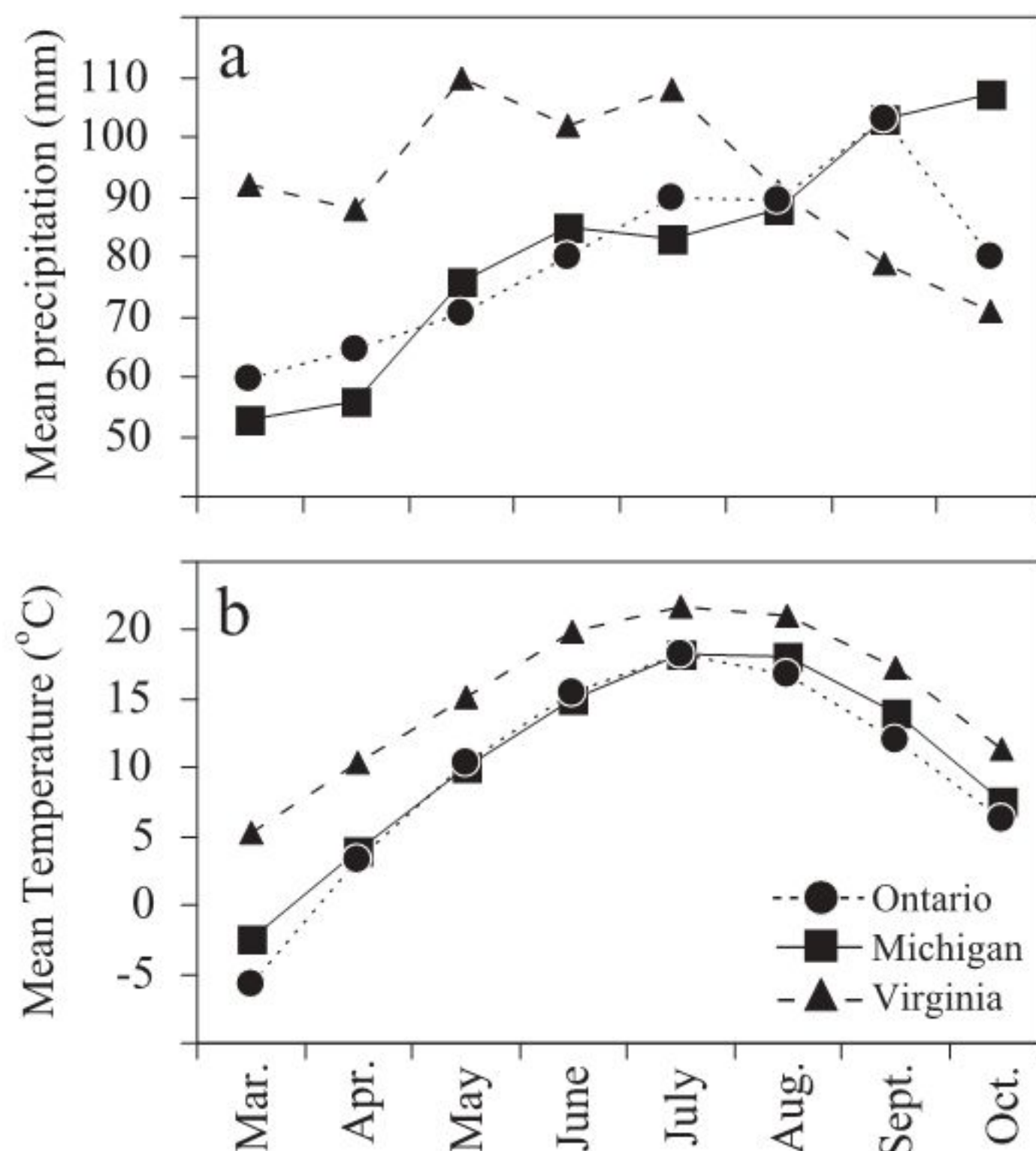
### General discussion

The present study cannot definitively conclude whether local *P. cinereus* are territorial, but the weight of evidence suggests that true territoriality may not be exhibited in the populations we examined. The main evidence against territoriality is, first, that a random spatial distribution in the field was temporally persistent at the six plots we examined and this distribution extended into early summer. Second, aggressive behaviour did not differ between resident and intruding salamanders and the amount of time spent in ATR behaviour was much lower than observed in many other populations. If territorial behaviour is conceptualized as an extreme or endpoint along a continuum of social behaviour and spacing patterns, then the evidence suggests that local populations likely fall short of this endpoint while nevertheless expressing some characteristics of territorial populations. As we describe below, this suggestion is not without precedent.

Previous studies on the Seal Salamander (*Desmognathus monticola* Dunn, 1916), a plethodontid salamander, suggest that individuals of this species defend and expel intruders from a foraging area (Keen and Reed 1985). However, this behaviour does not coincide with site fidelity and as such it does not constitute true territoriality (Mathis et al. 1995). Similar behaviour is observed in the Eurasian Reed Warbler (*Acrocephalus scirpaceus* (Hermann, 1804)), where individuals do not engage in the defense of a permanent area, rather they defend the area in which they happen to be foraging (Davies and Green 1976). We suggest that local *P. cinereus* might also defend a specific area, but that this area is transient. To this end, it is interesting that we observed a weak but general propensity towards uniform spatial distributions, which would appear to be congruent with low levels of aggression in the defense of transient feeding areas. This suggestion is tentative, however, and must be validated by an assessment of site tenacity in the field, as site tenacity was not assessed in the present study.

If we are correct in our suggestion that local populations are not truly territorial, then what factors differ between Ontario and MLBS, where individuals appear to be territorial? Similarly, why do individuals exhibit uniform spatial distributions at MLBS, whereas individuals sometimes aggregate in Michigan (Quinn and Graves 1999)? Given the limited data on geographic variation in territoriality of *P. cinereus*, it would at the present be premature to arrive at any conclusion. Indeed, a wide range of ecological and social factors can influence aggressive behaviour in *P. cinereus*, including the elevation of the population (Wise 1995), time of year (Jaeger 1979), the presence of interspecific competitors in the environment (Wise 1995), and even social learning (Liebgold 2014). Nevertheless, the present study may be able to provide insight into some general patterns. For instance, prey may be more readily available for salamanders on the surface than in underground burrows (Fraser 1976), but the surface activity of *P. cinereus* is limited by moisture (Jaeger 1980). If salamanders can profit more by remaining above ground during short intervals of dry weather, then it may be economical to defend a fixed territory (Jaeger 1980), although retreating underground may become unavoidable when summer temperatures reach a maximum. In this paradigm, the frequency and amount of precipitation during the spring and summer will help determine the amount of moisture present in the environment and it may be a general predictor of territoriality (Jaeger 1972, 1980; Ovaska 1988; Jaeger and Forester 1993). Interestingly, a broad comparison of precipitation patterns that occur near the study sites in Michigan, Virginia, and Ontario reveals that mean monthly precipitation is generally greater at MLBS during the majority of the growing season (Figs. 4a, 4b), which is the opposite of the predicted pattern. Yet it is perhaps not surprising that broad patterns of precipitation alone do not appear to coincide with the territorial behaviour of *P. cinereus*, as it is likely the interaction of precipitation patterns, temperature, and other local environmental factors that predict moisture on the forest floor.

**Fig. 4.** Climate normals for three locations near respective Eastern Red-backed Salamander (*Plethodon cinereus*) study sites or collection areas: Crystal Falls, Ontario, Canada (46°27'00"N, 79°52'00"W); Munising, Michigan, USA (46°24'43"N, 86°39'45"W); Blacksburg, Virginia, USA (37°12'06"N, 80°24'48"W). (a) Mean monthly precipitation and (b) mean monthly temperature. The active season in Virginia spans approximately April to November, whereas the active season in Michigan and Ontario spans approximately May to the end of October.



One possibility is that it might be generally uneconomical for *P. cinereus* to adopt territorial behaviour in cooler climates, as metabolic costs may generally be lower, and relatively low evapotranspiration rates might relax the constraint on foraging activity imposed by moisture. This might help explain the lack of territoriality in Michigan and potentially in Ontario, although this broad explanation would not address the different spatial distributions exhibited in these two areas. Furthermore, this conjecture is not entirely consistent with the findings of Wise (1995), where a comparison of eight populations of *P. cinereus* demonstrated that aggression was generally lower in populations from low elevation, compared with high elevation, suggesting temperature might not be as important as we have tentatively proposed.

We emphasize that very localized differences in resource quality and prey abundance might drive differences in territorial behaviour over fine spatial scales (Maerz and Madison 2000) and that the extent of territorial behaviour varies within a given growing season (e.g., Jaeger 1979). We have not intended to imply that territoriality is some fixed property of a population, as it can clearly be adopted when it is economical to do so (Davies 1976), provided the appropriate behavioural and physiological prerequisites exist (e.g., a mechanism of territorial advertisement, a propensity for aggressive behaviour). Nevertheless, these microgeographic and temporal differences in territorial behaviour are likely to be driven by corresponding differences or changes in the social or physical environment, and as such, it remains possible that there exists a general relationship between climate variables (or combinations of climate variables) and territorial behaviour across the range of this species.

In conclusion, the present study communicates two major insights. First, while territorial behaviour of *P. cinereus* is well documented at MLBS, territorial behaviour of *P. cinereus* in general should not be assumed and the prerequisites of territorial behaviour should be tested before inference is made with respect to spacing system. Second, evidence from the present study and from that of Quinn and Graves (1999) tentatively suggest that at least two northern populations of *P. cinereus* may not be territorial. These two studies underline the possibility of a more general pattern: an absence of true territoriality in more northern populations. On the whole, our understanding of the general factors that drive the evolution of territoriality in *P. cinereus* is limited by a paucity of studies that investigate geographic variation in the territorial behaviour of this species. A better understanding might be achieved if future studies test the prerequisites of territoriality from a wider variety of northern and southern populations.

### Acknowledgements

We thank A. Sugar and the Ministry of Natural Resources and Forestry for generously providing data and access to their sampling plots; our endeavor would not have been possible without their support. We thank our field research assistants D. Reeves, J. Crowley, K. Cowcill, S. Langlois, and J. Horkey. We are also indebted to J. Maerz, B. Graves, M. Peterson, H. Wilbur, A. Mathis, and two anonymous reviewers. Their correspondences, comments, and advice improved our experimental design and made this project possible. Finally, we thank S. Wise for providing a copy of her thesis. Animal care protocols were approved by the Nipissing University Animal Care Committee.

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